MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2022

July–September 2022—Volume 137, pp. 521–536

https://doi.org/10.5248/137.521

Texas microfungi: on the taxonomic placement of *Flosculomyces floridaensis* in *Zygosporiaceae*

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Abstract—The phylogenetic relationships and taxonomic placement of the anamorph *Flosculomyces floridaensis* are explored for the first time based on a strain isolated from a culturable air sample collected indoors in Texas, USA. Unpublished sequences obtained online from six well-characterized strains isolated in Japan were also included. Phylogenetic analyses using DNA sequence data from two different nuclear ribosomal loci (ITS, LSU) suggest that the fungus is a member of *Xylariales* (*Sordariomycetes*) and forms a distinct monophyletic lineage within *Zygosporiaceae*. The genus is recognized as a phylogenetically well-circumscribed taxon in agreement with its peculiar and unique morphology. The monophyletic *Zygosporiaceae* is recovered as five distinct and well delimited lineages based on the disparate morphologies of their anamorphs whereas *Zygosporium* was resolved as paraphyletic within the family. *Flosculomyces floridaensis* has not previously been reported in the continental USA outside its type locality in Florida and is recorded here for the first time from Texas.

Key words—airborne, hyphomycetes, taxonomy

Introduction

Flosculomyces B. Sutton was introduced to accommodate a peculiar anamorph collected on dead leaves of *Podocarpus* sp. (*Podocarpaceae*) in subtropical Florida, U.S.A. (Sutton 1978). Its type species, *F. floridaensis* B. Sutton, is characterized by round, four-celled, lobed and cruciately septate conidia that are darkly pigmented in the center, paler toward the periphery, and horizontally flattened in lateral view. They emerge from

holoblastic, integrated, or discrete conidiogenous cells, usually ampulliform and constricted in the middle, their apices often collapsing and becoming cupulate after conidium detachment. They arise singly or disposed on lateral branches with one or two orders, usually in whorls of up to three at the apex of macronematous, mononematous conidiophores. A second species, *F. trilobatus* Onofri, was later described on dead leaves collected in Ivory Coast (Onofri 1984), with slightly smaller, three-celled conidia. The genus is considered *Ascomycota* incertae sedis (Wijayawardene & al. 2021) and its phylogenetic relationships are currently unknown. However, Tanaka & al. (2017) placed *F. floridaensis* in *Xylariales* based on BLAST searches of ITS sequence data obtained from a strain isolated in Japan.

The fungus apparently has a pantropical distribution (Mena & al. 2020) with records mainly in the Neotropics and the Asian Paleotropics. In the Neotropics, it has been recorded several times in Brazil on dead leaves of *Clusia nemorosa* G. Mey. (*Clusiaceae*) (Marques & al. 2007, Barbosa & al. 2009) and in Cuba on a branch of *Pandanus* sp. (*Pandanaceae*) (Arnold 1985), dead rachides of *Cocos nucifera* L. (*Arecaceae*) (Mercado & al. 2002), dead leaves of *Calophyllum antillanum* Britton, *C. calaba* L. (*Calophyllaceae*), and *Pimenta dioica* (L.) Merr. (*Myrtaceae*) (Camino & al. 2006), and petioles of dead leaves of *Roystonea regia* O.F. Cook (*Arecaceae*) (Mena & al. 2020). In the Paleotropics, Yokoyama & Ito (1989) first isolated the fungus on culture media from peduncle sheath of *Pandanus boninensis* Warb. and fallen leaves of *Calophyllum inophyllum* L. and *Lithocarpus edulis* Nakai (*Fagaceae*) in Japan. The resulting four strains are currently deposited in NBRC (http://www.nite.go.jp/en/nbrc/cultures/index.html). Other strains were obtained in Japan from leaves of *Pittosporum tobira* W.T. Aiton (*Pittosporaceae*) by Ono & Kobayashi (2005) and from leaves of *Arenga engleri* Becc. (*Arecaceae*) by Tanaka & al. (2017). These two strains are currently deposited in MAFF and accessible at the NARO GenBank database (https://www.gene.affrc.go.jp/databases_en.php). Additional records have been reported from Hong Kong on decaying leaves of *Pandanus furcatus* Roxb., the Philippines on decaying leaves of *Pandanus* sp. (Whitton & al. 2012), and India as an endophyte from fresh leaves and twigs of *Artocarpus* sp. (*Moraceae*) (Bhat 2010). There are also scattered records in Africa on leaves of *Pandanus* sp. in Mauritius (Dulymamode & al. 2001) and on dead leaves of *Drypetes aylmeri* Hutch. & Dalziel (*Putranjivaceae*) and *Didelotia idae* J. Léonard & al. (*Fabaceae*) in Ivory Coast (Rambelli & al. 2004). Matsushima (1989), on the other hand, reported the fungus on dead leaves of *Acacia aulacocarpa* A. Cunn. ex Benth.

(*Fabaceae*) in Australia. Frequently, airborne conidia of *F. floridaensis* are found in the atmosphere suggesting that wind plays an important role in their dispersal. Although originally described from Florida, the fungus has not been further reported in other locations across the continental USA.

During routine analyses of culturable air samples received in our laboratory for indoor air quality assessments, a colony of *F. floridaensis* was identified and recovered. In order to determine the phylogenetic relationships of the fungus and to infer its taxonomic placement within *Ascomycota*, DNA sequence data of two different nuclear ribosomal loci were obtained and analyzed. Results are presented here along with morphological and cultural studies of the recovered strain and couple of non-culturable airborne specimens. They serve to document the presence of the fungus in Texas.

Materials & methods

Isolation and morphological studies

The original colony was recovered from an indoor air sample collected on an agar plate using an Andersen sampler (Andersen 1958) after 7 days of incubation at 25°C. Single conidia were transferred aseptically to 2% Malt Extract Agar (MEA) and incubated following identical conditions. Colony features were observed after seven days with sporulation being monitored during subculturing. Microscopic slides were mounted in lacto-cotton blue and microphotographs were taken using an Olympus BX-45 compound microscope. A total of 50 measurements were made at 1000× magnification for each fungal structure. Minimum, maximum, 5th and 95th percentile values were calculated with outliers given in parentheses. Unfortunately, it was not possible to deposit a living culture due to persistent contamination following subculturing. However, a clean voucher specimen, in the form of a dried culture and semi-permanent slides, is deposited in the U.S. National Fungus Collections, Beltsville MD, USA (BPI). Non-culturable airborne conidia collected on glass slides inside Air-O-Cell sampling cassettes (Zefon International, Ocala, Florida, USA) were also studied following similar procedures and slide specimens were deposited as well in BPI. Fungal names throughout the text follow Index Fungorum (http://www.indexfungorum.org/) and Tropicos (https://tropicos.org/home) for host plant names. Herbaria acronyms follow Index Herbariorum (http://sweetgum.nybg.org/science/ih/) and culture collection acronyms follow the Culture Collections Information Worldwide of the WFCC-MIRCEN World Data Center for Microorganisms (http://www.wfcc.info/ccinfo/).

DNA extraction, PCR amplification, and sequencing

An isolate was grown on MEA for 14 days at 25°C and sent to a sequencing facility, Laragen, Inc. for Microbial Identification Service (http://www.laragen.com/laragen_microbial.php). Genomic DNA was extracted from fresh mycelium following the company's protocols and the complete internal transcribed spacer

Table 1. Strains and sequences included in the phylogenetic analysis. New sequences are highlighted in bold.

Taxon	Strain	GenBank ITS	GenBank LSU
Ascotricha chartarum	CBS 104.25	MH854797	MH866301
	CBS 110.52	MH856946	MH868472
	CBS 902.69	MH859477	MH871257
	HMCK-30	MK051122	MK051166
A. erinacea	CBS 116.31	MH855151	MH866600
	CBS 194.71^{T}	MH860063	MH871844
	CBS 407.87	KF893287	—
	CBS 535.73	KF893285	MH872482
	NBRC 32298^{T}	IF03229801*	IF03229801*
	NBRC 9841	IF00984101*	IF00984101*
A. funiculosa	CBS 124.80^{T}	MH861246	MH873018
	CBS 323.86	KU683762	KU683762
A. lusitanica	CBS 462.70^{T}	KF893289	MH877806
	FMR 17607	—	LR812705
A. microspora	NHES L1706^{T}	MF805818	—
A. pusilla	CBS 132.60	MH857921	MH869465
A. rugispora	NBRC 33167^{T}	LC146763	LC146763
Ascotricha sp.	GLMC 453	MT153622	MT156161
Astrocystis sublimbata	CBS 130006	MH865618	MH877041
Atrotorquata spartii	MFLUCC 13-0444	—	KP325443
Ciliosporella italica	MFLU 16-1114^{T}	MT177929	MT177956
Coniocessia anandra	IRAN 1468C^{T}	GU553338	GU553349
C. cruciformis	IRAN 1474C	GU553335	GU553346
C. maxima	IRAN 1602C	GU553332	GU553344
C. nodulisporioides	CBS 281.77	GU553342	AJ875224
Entoleuca mammata	CBS 235.34	—	MH866995
Flosculomyces floridaensis	BPI 919995	**MZ353014**	**MZ353015**
	MAFF 239738	—	—
	MAFF 243957	—	—
	NBRC 30653	IF03065301*	IF03065301*
	NBRC 30654	IF03065401*	IF03065401*
	NBRC 30655	IF03065501*	IF03065501*
	NBRC 30656	IF03065601*	IF03065601*
Guayaquilia cubensis	MUCL 39017	KC775733	KC775708
Hypocopra rostrata	NRRL 66178	KM067909	KM067909
Idriella lunata	CBS 204.56^{T}	KP859044	KP858981
Kretzschmaria deusta	CBS 723.69	—	MH871168

Microdochium majus	CBS 741.79	KP859001	KP858937
M. nivale	CBS 116205	KP859008	KP858944
Nemania serpens	CBS 241.65	—	MH870193
Neoidriella desertorum	CBS 985.72[T]	KP859048	KP858985
Paraxylaria rosacearum	TASM 6132[T]	MG828941	MG829050
Rosellinia aquila	ILLS 121141	—	MG845555
R. convexa	MFLUCC 19-0469	MN707567	MN707569
R. corticium	E32_II	KC311485	KC311485
R. necatrix	CBS 267.30	KF719201	MH866585
Selenodriella fertilis	CBS 772.83	KP859055	KP858992
Sordaria fimicola	CBS 723.96	MH862606	MH874231
Triangularia verruculosa	CBS 433.64	MH858480	MH870112
Vesiculozygosporium echinosporum	CBS 247.72	MH860466	—
	CPC 35607	MT223869	MT223940
	JCM 11115	AB073533	AB073534
Wawelia regia	CBS 1100	MH854595	MH866123
Xylaria longipes	DSM 107183	MK408619	MK408619
Zygosporium chartarum	CBS 384.53	—	MH868785
Z. gibbum	FMR 13130	KY853482	KY853546
	NBRC 30213	IF03021301*	IF03021301*
Z. masonii	CBS 138.71	MH860038	MH871818
	CBS 479.73	MH860748	MH872461
	CBS 557.73	MH860771	MH872493
	NBRC 30214	IF03021401*	IF03021401*
Z. minus	HKAS 99625	MF621586	MF621590
Z. mycophilum	CBS 396.49	MH856563	MH868080
	CBS 533.76	—	MH877824
	CBS 828.68	—	MH878406
	CBS 894.69	MH859474	MH871255
	NBRC 32050	IF03205001*	IF03205001*
	NBRC 9359	IF00935901*	IF00935901*
Z. oscheoides	CBS 195.79	MH861194	MH872964
	MFLUCC 14-0402	MF621585	MF621589
Z. pseudogibbum	CBS 143503[T]	MH107928	MH107974
	CBS 144442	MK442633	MK442568
Z. pseudomasonii	CPC 37503[T]	MN562147	MN567654

[T] Strains with type status

*Sequences and their accession numbers downloaded from the Biological Resource Center (NBRC), NITE, Japan (http://www.nite.go.jp/en/nbrc/cultures/index.html)

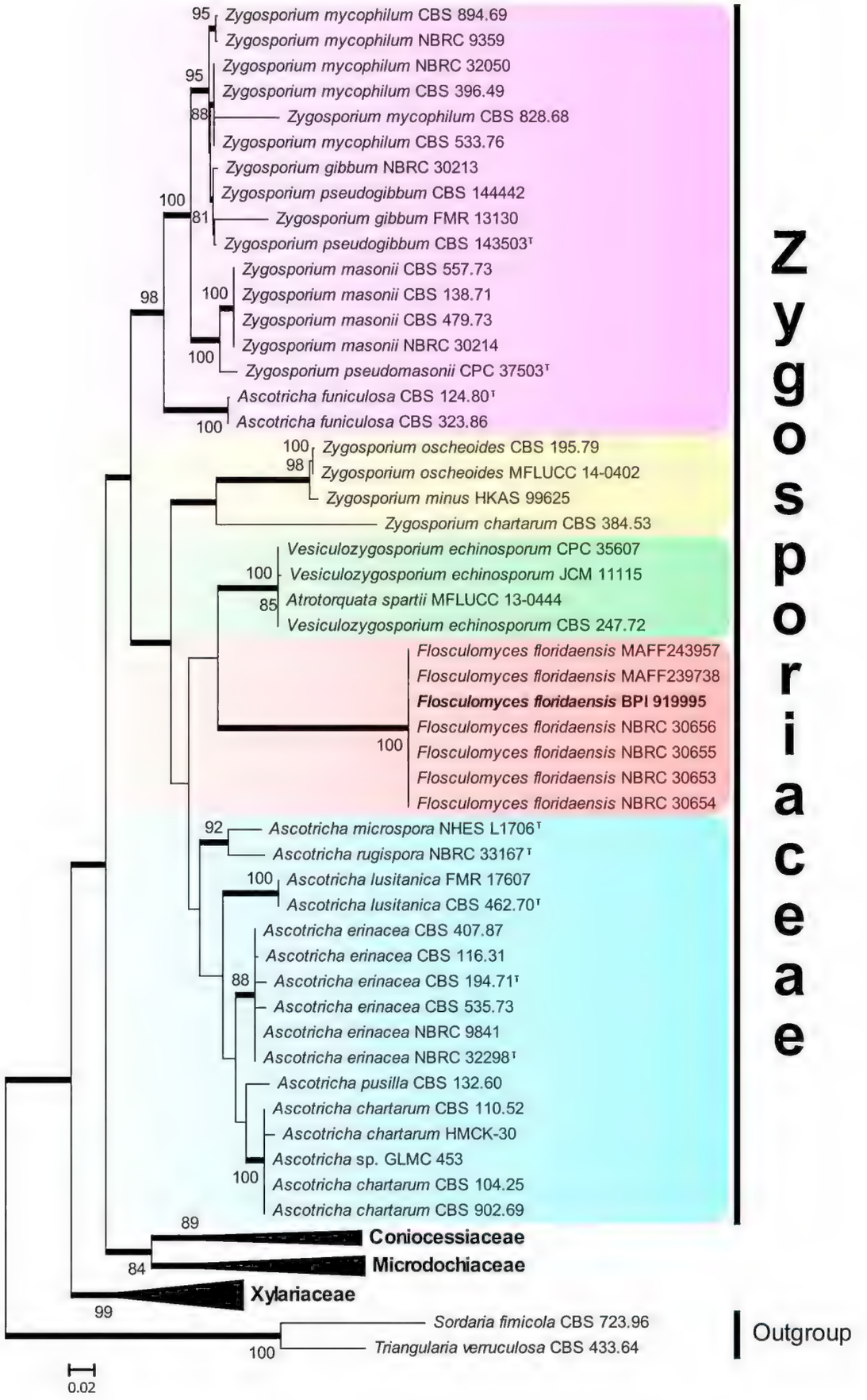
** Sequences downloaded from NARO Genebank database without accession numbers (https://www.gene.affrc.go.jp/databases_en.php)

(ITS) region together with the first 900 bp of the 5′ end of the nuclear ribosomal large subunit DNA (nrLSU) were PCR amplified, purified, and sequenced using the primers pairs ITS1/ITS4 and NL1/LR5 (O'Donnell 1992, Vilgalys & Hester 1990, White & al. 1990). The returned consensus sequences were checked and deposited in GenBank.

Taxon sampling and phylogenetic analyses

The newly generated ITS and LSU sequences were first subjected to megablast searches in GenBank to assess the phylogenetic placement of *F. floridaensis*. Closest hits were members of *Zygosporiaceae* and *Xylariaceae* (*Xylariales*), which were selected and used to assemble datasets for analyses. Unpublished ITS–LSU sequences belonging to six strains of *F. floridaensis* collected in Japan (Tanaka & al. 2017, Yokoyama & Ito 1989) were retrieved from the NBRC and NARO Genetic Resources Center websites and added to the datasets. Additional sequences belonging to recent phylogenies of *Zygosporiaceae* and related families (Li & al. 2017, Li & Zhao 2018, Crous & al. 2020, Konta & al. 2021) were also included. The strains *Sordaria fimicola* CBS 723.96 and *Triangularia verruculosa* CBS 433.64 (*Sordariales*) were selected as outgroup. All strains and sequences used in this study are listed in Table 1. Individual ITS and LSU datasets were aligned separately using MAFFT v 7.487 on the online server (Katoh & al. 2002), which automatically selected the FFT-NS-i strategy (Katoh & Standley 2013) for both loci. Phylogenetic reconstructions were first performed on individual datasets. Maximum likelihood (ML) was employed using RAxML v.8.2.10 (Stamatakis 2014) and Bayesian inference (BI) using MrBayes v. 3.2.7a (Ronquist & Huelsenbeck 2003) on the CIPRES Science Gateway server (Miller & al. 2010). Each analysis setting followed Delgado & al. (2022) with statistical support estimated for each node using bootstrap (BS) and posterior probabilities (BPP). Some topological differences existed between individual trees but recovered clades were identical and well supported. Therefore, datasets were concatenated in MEGA v. 6.06 (Tamura & al. 2013) for further ML and BI analyses following identical settings. Pairwise comparisons and best-fit substitution model selection were also performed in MEGA. The selected model for BI using the Akaike information criterion was GTR+G+I for all datasets. Trees were viewed in FigTree v1.4.2. (Rambaut 2009) or MEGA and edited on Inkscape (inkscape.org).

Fig. 1. RaxML phylogenetic tree inferred from combined ITS–LSU nrDNA sequences of *Zygosporiaceae* and related taxa in *Xylariales* showing the placement of *F. floridaensis* within the family. The new strain obtained in this study is in bold. Color boxes represent the five different lineages recovered: *Zygosporium* sensu lato (purple); *Zygosporium* sensu stricto (yellow); *Vesiculozygosporium* (green); *Flosculomyces* (pink) and *Ascotricha* (pale blue). Bootstrap support values ≥70% are shown at the nodes and Bayesian posterior probabilities ≥0.95 are indicated by thickened branches. [T] denotes strains with type status.


Zygosporium mycophilum CBS 894.69
Zygosporium mycophilum NBRC 9359
Zygosporium mycophilum NBRC 32050
Zygosporium mycophilum CBS 396.49
Zygosporium mycophilum CBS 828.68
Zygosporium mycophilum CBS 533.76
Zygosporium gibbum NBRC 30213
Zygosporium pseudogibbum CBS 144442
Zygosporium gibbum FMR 13130
Zygosporium pseudogibbum CBS 143503T
Zygosporium masonii CBS 557.73
Zygosporium masonii CBS 138.71
Zygosporium masonii CBS 479.73
Zygosporium masonii NBRC 30214
Zygosporium pseudomasonii CPC 37503T
Ascotricha funiculosa CBS 124.80T
Ascotricha funiculosa CBS 323.86
Zygosporium oscheoides CBS 195.79
Zygosporium oscheoides MFLUCC 14-0402
Zygosporium minus HKAS 99625
Zygosporium chartarum CBS 384.53
Vesiculozygosporium echinosporum CPC 35607
Vesiculozygosporium echinosporum JCM 11115
Atrotorquata spartii MFLUCC 13-0444
Vesiculozygosporium echinosporum CBS 247.72
Flosculomyces floridaensis MAFF243957
Flosculomyces floridaensis MAFF239738
Flosculomyces floridaensis BPI 919995
Flosculomyces floridaensis NBRC 30656
Flosculomyces floridaensis NBRC 30655
Flosculomyces floridaensis NBRC 30653
Flosculomyces floridaensis NBRC 30654
Ascotricha microspora NHES L1706T
Ascotricha rugispora NBRC 33167T
Ascotricha lusitanica FMR 17607
Ascotricha lusitanica CBS 462.70T
Ascotricha erinacea CBS 407.87
Ascotricha erinacea CBS 116.31
Ascotricha erinacea CBS 194.71T
Ascotricha erinacea CBS 535.73
Ascotricha erinacea NBRC 9841
Ascotricha erinacea NBRC 32298T
Ascotricha pusilla CBS 132.60
Ascotricha chartarum CBS 110.52
Ascotricha chartarum HMCK-30
Ascotricha sp. GLMC 453
Ascotricha chartarum CBS 104.25
Ascotricha chartarum CBS 902.69
Coniocessiaceae
Microdochiaceae
Xylariaceae
Sordaria fimicola CBS 723.96
Triangularia verruculosa CBS 433.64
Zygosporiaceae
Outgroup
0.02

Phylogenetic results

Pairwise comparisons between the ITS and LSU sequences of the Texas and Japanese strains of *F. floridaensis* showed they were identical along the lengths of their 483 ITS and 560 LSU aligned positions. The combined ITS–LSU alignment consisted of 77 sequences (including the outgroup sequences) and 1522 positions—627 from the ITS and 895 from the LSU datasets. The best scoring RAxML tree (ln = −12408.076162) is shown in Fig. 1. The tree was identical in topology to the 50% majority rule consensus tree of the 12001 trees sampled during the Bayesian analysis. The seven strains of *F. floridaensis* formed a strongly supported monophyletic group (the *Flosculomyces* lineage; 100% BS, 1 BPP) within *Zygosporiaceae* J.F. Li & al. They grouped sister to another strongly supported clade (100% BS, 1 BPP), including three strains of *Vesiculozygosporium echinosporum* (Bunting & E.W. Mason) Crous and another strain named *Atrotorquata spartii* Thambug. & al. (the *Vesiculozygosporium* lineage), but without significant support. *Ascotricha* strains grouped together in a weakly supported clade (the *Ascotricha* lineage) with the exception of the two available strains of *A. funiculosa* (Guarro & Calvo) D.W. Li & G.H. Zhao including an ex-type CBS 124.80. Both strains were recovered as sister to the fully supported *Zygosporium* sensu lato lineage (100% BS, 1 BPP), including species such as *Z. mycophilum* (Vuill.) Sacc., *Z. gibbum* (Sacc. & al.) S. Hughes, and *Z. masonii* S. Hughes, with strong support (98% BS, 0.99 BPP). The *Zygosporium* sensu stricto clade including *Z. oscheoides* Mont. (the generic type), received significant support only in the Bayesian analysis (0.99 BPP). It was resolved sister to a subclade formed by *Vesiculozygosporium*, *Flosculomyces,* and *Ascotricha* lineages with significant BPP support (0.99). *Zygosporiaceae* was recovered as a monophyletic clade but having only significant Bayesian support (0.98 BPP). Some topological incongruences were observed between individual trees. They include the placement of the *Flosculomyces* clade outside *Zygosporiaceae* in the ITS tree or the position of *A. rugispora* NBRC 33167 outside the *Ascotricha* clade in the LSU tree (not shown). However, the different lineages recovered were identical and strongly or similarly supported in all analyses.

Taxonomy

Flosculomyces floridaensis B. Sutton, Mycologia 70(4): 789, 1978. Fig. 2

Colonies on MEA moderately fast growing reaching 22–24 mm diam. after 7 days at 25°C, velvety, white, center cream or dull white, flat, sometimes slightly sulcate, margin entire, reverse dull white, readily sporulating at

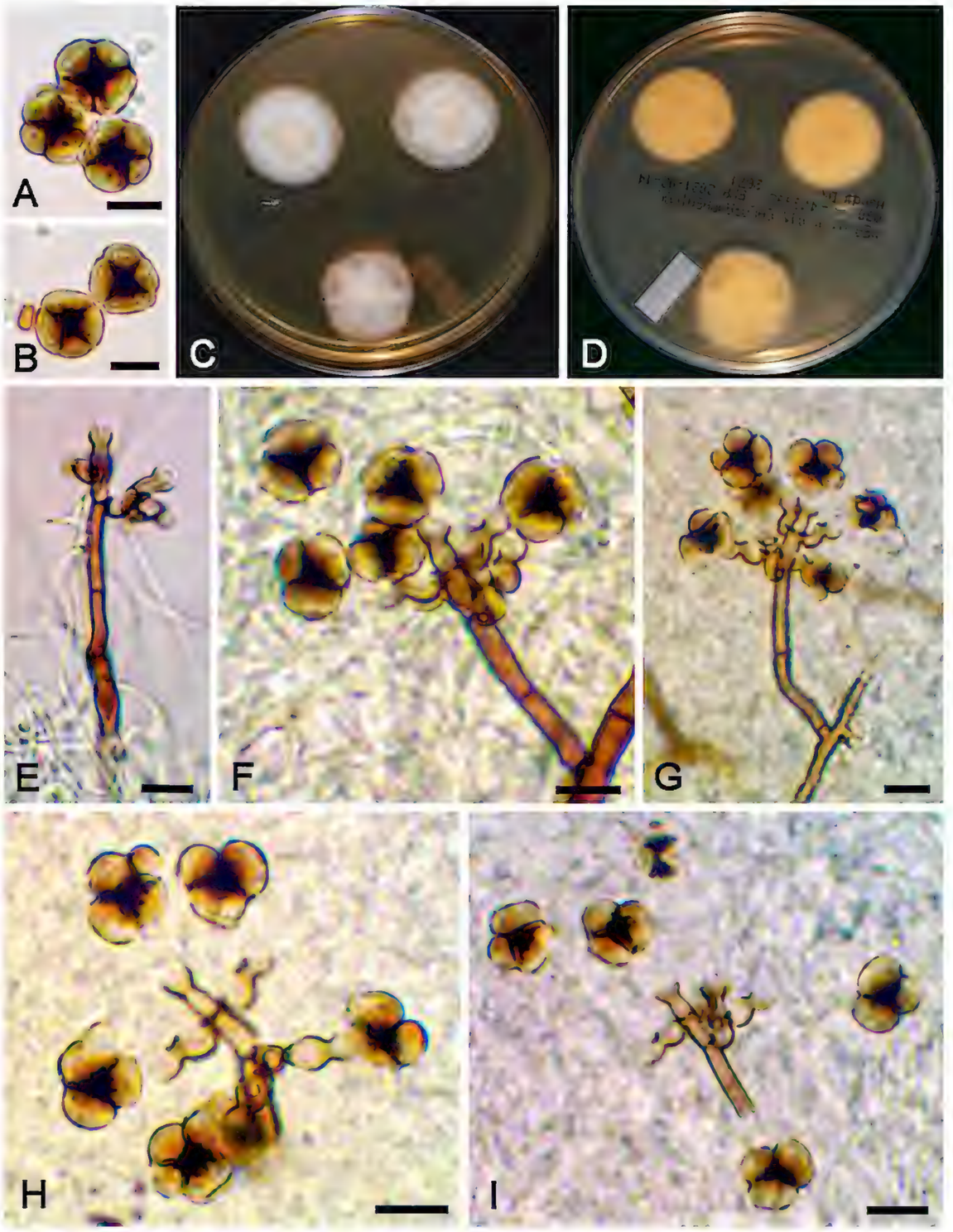


Fig. 2. *Flosculomyces floridaensis* (BPI 928572): A, B. Airborne conidia (BPI 919995); C, D. Colonies on MEA after 7 days at 25°C (C. Surface view; D. Reverse); E. Conidiophore with apical branches and conidiogenous cells; F, G. Conidiophores, branches, conidiogenous cells, and conidia; H, I. Apical branches, conidiogenous cells; and mostly 3-celled conidia. Scale bars = 10 μm.

first but declining to almost sterile after repeated subculturing. Mycelium immersed and superficial, composed of branched, septate, smooth, hyaline immersed hyphae, 1.5–2 µm wide, subhyaline to pale brown in mass, and smooth or irregularly verruculose, pale brown to brown, superficial hyphae, (2–)3–5 µm wide, some cells or segments thick-walled and swollen, 4.5–7 µm wide. Conidiophores arising terminally or laterally on the superficial hyphae, macronematous, mononematous, erect, straight or flexuous, branched toward the apex or rarely simple, septate, smooth, brown, up to 121 µm long, 3–4 µm wide; branches intercalary and lateral on the conidiophores, formed below the septa, single or in whorls of 2–3, pale brown to brown, subcylindrical, sometimes slightly swollen at the base, 6–9(–12) × 4–6 µm wide. Conidiogenous cells monoblastic, determinate, integrated and terminal or intercalary and lateral on the conidiophores or discrete on lateral branches, ampulliform, constricted in the middle, 6–10 µm long, 3–5 µm at the base, rounded at the apex, brown, paler toward the apex, sometimes cupulate after conidial detachment, 3.5–6 µm wide at the apex. Conidia acrogenous, consisting of three or four cells, more or less round, lobed, cruciately septate, constricted at the septa, smooth, brown, darkly pigmented in the center, paler toward the periphery, 10–16 µm diam., horizontally flattened and black at base in lateral view, 6–7 µm thick.

Specimens examined—**UNITED STATES. Texas: Montgomery Co.**, The Woodlands 77384, 30.2051°N 95.4582°W, from indoor air collected using a pre-cultured MEA plate, isol. G. Delgado, 9.XII.2020 (BPI 919995); **Harris Co.**, Houston 77096, from outdoor air collected on a glass slide inside an Air-O-Cell sampling cassette, 28.V.2021, det. G. Delgado (BPI 928571); Kingwood 77345, from outdoor air collected on a glass slide inside an Air-O-Cell sampling cassette, 3.VI.2021, det. G. Delgado (BPI 928572).

Comments—Our isolate and the other two specimens consisting of only airborne conidia agree well with the species protologue in morphology and conidial dimensions, 13–16.5 µm diam. and 7–8 µm thick. However, a mixture of conidia (predominantly 3-celled) was present in the cultured isolate (Fig. 2H, I), whereas 4-celled conidia dominated the airborne samples (Fig. 2A, B). The presence of both three or four-celled conidia in *F. floridaensis* specimens has been previously reported in culture or natural substrate. Matsushima (1989) mentioned the production of conidia having both three or four lobes on corn meal and V8 agar media. Yokoyama & Ito (1989) and Ono & Kobayashi (2005) described conidia usually consisting of four or (rarely) three cells, whereas Marques & al. (2007) mentioned both smaller, 3-celled conidia and slightly larger, 4-celled conidia for materials

collected from the same substrate and locality. Apparently, age and growing conditions, whether on the host, indoor, subject to air dispersion, or in culture, influence the production of conidia with a greater or fewer number of cells. This may suggest that morphological boundaries between the two accepted species, mainly separated by the number of conidial cells, are fuzzy and the validity of *F. trilobatus* needs to be tested in the light of molecular data. In culture, our isolate matched well those of Yokoyama & Ito (1989) in being white at first, grayish salmon color with age on MEA. Unfortunately, successive sub-culturing affected its sporulation and the Texas isolate decreased capacity to the point of becoming nearly sterile after two months.

Discussion

In the present study, the taxonomic placement of *Flosculomyces floridaensis* is investigated for the first time based on nuclear ribosomal DNA sequence data obtained from strains collected in Japan and the USA. The position of the fungus in *Zygosporiaceae* (*Xylariales*, *Sordariomycetes*) revealed a novel and distinct lineage within the family. *Flosculomyces* is therefore recognized as a phylogenetically well-circumscribed genus in agreement with its peculiar and unique morphology. Based on ribosomal DNA sequence data and strains collected in Thailand, China, and Spain, Li & al. (2017) validly reintroduced the name *Zygosporiaceae* to accommodate three species of *Zygosporium* Mont.: *Z. oscheoides* (the generic type), *Z. minus* S. Hughes, and *Z. gibbum*. The genus forms a well-supported monophyletic lineage within the *Xylariales* sister to *Coniocessiaceae* Dania García & al. and molecular results were also consistent with the unique morphology of its members. *Zygosporium* is characterized by hyphomycetous anamorphs having darkly pigmented, incurved vesicular cells that may be stalked or sessile and borne from the side of a setiform conidiophore or arise directly from the mycelium giving rise to 2–4 ampulliform conidiogenous cells producing aseptate, typically ellipsoid or globose, smooth or variously ornamented conidia (Whitton & al. 2003, 2012). Later, sequence data for additional species such as *Z. chartarum* Camposano, *Z. masonii,* and *Z. mycophilum* became available in GenBank for strains deposited in CBS (Vu & al. 2019). Two novel species, *Z. pseudomasonii* Crous and *Z. pseudogibbum* Crous, were also described using DNA sequence data (Crous 2019, Crous & al. 2018, 2019) together with a new genus *Vesiculozygosporium* Crous based on *Z. echinosporum* Bunting & E.W. Mason (Crous & al. 2020). On the other hand, the holomorphic genus *Ascotricha* Berk. has been traditionally accepted within *Xylariaceae* (Cheng & al. 2015,

Wijayawardene & al. 2020). However, with the availability of DNA sequence data for *Zygosporium* species, Hyde & al. (2020) recently recognized the close phylogenetic affinities between these two genera. Subsequent studies have included *Ascotricha* within *Zygosporiaceae* distant from *Xylariaceae* (Crous & al. 2019, 2020) and our phylogeny was also consistent with these results.

With the placement of *Flosculomyces* in *Zygosporiaceae*, the family apparently includes disparate morphologies based on their anamorphs that can be easily recognized as five distinct and well delimited lineages (Fig. 1). The typical vesiculate, setiform conidiophores and aseptate, ellipsoidal or globose conidia of the *Zygosporium* and *Vesiculozygosporium* lineages contrast with the branched conidiophores and cruciately septate conidia of *Flosculomyces*. They are also dissimilar to the dichotomously or trichotomously branched conidiophores of the dicyma-like anamorphs of *Ascotricha*, having sterile, clavate extensions and sympodial, denticulate conidiogenous cells (Ellis 1971). Moreover, *Ascotricha* is so far the only member of *Zygosporiaceae* producing teleomorphic states. They are characterized by ostiolate or non-ostiolate, long setose ascomata with a translucent peridial wall, 8-spored asci and brown ellipsoidal ascospores with an equatorial germ slit (Stchigel & Guarro 1998, Cheng & al. 2015). Curiously, the sterile elements of setae and conidiophores in *Ascotricha* and *Zygosporium* suggest a morphological connection between them. The capitate, vesiculate or spathulate cells developing at the tips or at the geniculations and dichotomous branches of setae in the ascomata and conidiophores of *Ascotricha* species show some resemblance with the capitate, slightly swollen to spherical apical vesicles of setae and setiform conidiophores of *V. echinosporum*, *Z. minus* and *Z. oscheoides* (Hawksworth 1971, Hughes 1951). They also resemble the long, apically swollen or the short cylindrical sterile cells at the apex of the vesicular conidiophores of *Z. masonii*, *Z. gibbum*, and *Z. mycophilum*. From a morphological standpoint, these shared features may help to support further the recent placement of *Ascotricha* in *Zygosporiaceae*.

Interestingly, *Zygosporium* was resolved as paraphyletic within the family (Fig. 1). Morphologically, the genus was split according to whether or not vesicles are always produced at the side of the setiform conidiophores, and if they are not, whether vesicles are always or sometimes borne directly on the superficial mycelium accompanied by setae or without them. The *Zygosporium* sensu stricto lineage includes the generic type *Z. oscheoides*, together with *Z. chartarum* and *Z. minus*. The three species are characterized by the production of vesicles usually at the base of the setiform conidiophores.

On the other hand, the *Zygosporium* sensu lato lineage, at the moment the most specious one, includes *Z. gibbum*, *Z. pseudogibbum*, *Z. mycophilum*, *Z. masonii*, and *Z. pseudomasonii*. This group has in common the lack of setae and the presence of vesicles borne on stalk cells that arise directly on the superficial mycelium. The third lineage includes *Vesiculozygosporium* and consists of a single species, *V. echinosporum*, characterized by stalk vesicles either arising directly from the superficial mycelium or borne along the length of setiform conidiophores that sometimes are sterile, do not produce any vesicles, and therefore are considered true setae (Whitton & al. 2003, Li & al. 2017). A LSU sequence under the name *Atrotorquata spartii* MFLUCC 13-0444 clustered together with *V. echinosporum*. Crous & al. (2020) explained that there are inconsistences between this sequence and its ITS counterpart, and therefore it was only included in our phylogeny because of its 100% identity with the LSU sequence of strain CPC 35607. Further support for this lineage was obtained by adding strains CBS 247.72 and JCM 11115, the latter originally isolated from a nest of a termite *Odontotermes* sp. in Thailand as an unknown xylariaceous taxon (Taprab & al. 2002) but later identified as *V. echinosporum* (https://jcm.brc.riken.jp/en/catalogue_e). Additionally, the three lineages revealed in *Zygosporium* agree well with the morphological categories used in previous dichotomous keys to identify species of the genus (Whitton & al. 2003, 2012). According to Index Fungorum (http://www.indexfungorum.org), *Zygosporium* currently includes 28 species in contrast to only nine taxa currently available in GenBank. More strains representing other species and additional DNA sequence data will help to further test the hypothetical lineages revealed in this work for possible generic redisposition in the future.

Acknowledgments

Special thanks are due to Dr. De-Wei Li (The Connecticut Agricultural Experiment Station, USA) and Dr. Josiane S. Monteiro (Museu Paraense Emílio Goeldi, Brazil) for kindly serving as pre-submission reviewers of the manuscript. William Colbert and Dr. Kamash Pillai (Eurofins EMLab P&K, USA) are thanked for providing laboratory facilities. Dr. Lorelei Norvell and Dr. Shaun Pennycook are gratefully acknowledged for their tireless editorial work on behalf of Mycotaxon authors.

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